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DegS-DegU and ComP-ComA Modulator-Effector Pairs Control Expression of the *Bacillus subtilis* Pleiotropic Regulatory Gene *degQ*

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Production of a class of both secreted and intracellular degradative enzymes in *Bacillus subtilis* is regulated at the transcriptional level by a signal transduction pathway which includes the DegS-DegU two-component system and at least two additional regulatory genes, *degQ* and *degR*, encoding polypeptides of 46 and 60 amino acids, respectively. Expression of *degQ* was shown to be controlled by DegS-DegU. This expression is decreased in the presence of glucose and increased under any of the following conditions: growth with poor carbon sources, amino acid deprivation, phosphate starvation, and growth in the presence of decoyinine, a specific inhibitor of GMP synthetase. In addition, expression of *degQ* is shown to be positively regulated by the ComP-ComA two-component system. Separate targets for regulation of *degQ* gene expression by DegS-DegU and ComP-ComA were located by deletion analysis between positions −393 and −186 and between positions −78 and −40, respectively. Regulation of *degQ* expression by amino acid deprivation was shown to be dependent upon ComA. Regulation by phosphate starvation, catabolite repression, and decoyinine was independent of the two-component systems and shown to involve sequences downstream from position −78. The ComP-ComA and DegS-DegU two-component systems seem to be closely related, sharing several target genes in common, such as late competence genes, as well as the *degQ* regulatory gene. Sequence analysis of the *degQ* region revealed the beginning of an open reading frame directly downstream from *degQ*. Disruption of this gene, designated *comQ*, suggests that it also controls expression of *degQ* and is required for development of genetic competence.

Production of a class of degradative enzymes in *Bacillus subtilis*, including an intracellular protease and several secreted enzymes (levansucrase, alkaline and metalloproteases, α-amylase, β-glucanase[s], and xylanase) (3, 5, 11, 29), is controlled at the transcriptional level by the DegS-DegU two-component system and by at least two additional regulatory genes, *degQ* and *degR*, which encode small polypeptides of 46 and 60 amino acids, respectively (3, 25, 42, 48, 49).

Although the two-component system is required for degradative enzyme production, the products of *degQ* and *degR* appear to be dispensable (48, 49). Mutations were identified in both *degS* and *degU* leading to either deficiency of degradative enzyme production or a pleiotropic (Hy) phenotype, which includes hyperproduction of degradative enzymes, ability to sporulate in the presence of glucose, decreased genetic competence, and loss of flagella (6, 12, 17, 23).

Since degradative enzyme production requires both the DegS protein kinase and the DegU effector, we postulated that the phosphorylated form of DegU may be necessary for this process. On the other hand, we postulate that the nonphosphorylated form of DegU may be required for competence (23, 28; this report). This hypothesis is supported by the following observations: (i) the *degU146* mutation abolishing the putative site of phosphorylation of the DegU effector did not abolish competence (23), and (ii) deletion of the *degS* gene did not strongly reduce competence (this report).

The *degQ36* mutation, a single base change at position −10 leading to overexpression of the *degQ* gene, gave a Hy phenotype similar to that of the *degS*(Hy) and *degU*(Hy) mutations (3, 48). The increase of degradative enzyme production due to overproduction of DegQ is, however, strictly dependent upon the presence of a functional DegS-DegU two-component system (3; this report).

We previously reported that expression of *degQ* was decreased in strains carrying either a deletion of *degS* and *degU* or a *degU32*(Hy) mutation (23). In this report, we show that *degQ* expression is regulated by a second two-component system controlling competence in *B. subtilis*: ComP-ComA (46, 47). Several findings had suggested that this could be the case: (i) DegS-DegU and ComP-ComA show strong amino acid sequence similarities, (ii) both of these two-component systems control the expression of competence, and (iii) the *degQ*, *comP*, and *comA* genes are located at adjacent positions on the *B. subtilis* chromosome (47).

The DegS, DegU, and DegQ proteins are produced at different times during growth. The *degS-degU* operon is expressed throughout the exponential growth phase. The level of expression of *degQ*, however, is low during the exponential growth phase and was shown to increase substantially in the stationary phase, under conditions of carbon or phosphate source limitations, or during amino acid deprivation (23; this report).

MATERIALS AND METHODS

Strains. The *B. subtilis* strains used in this study are listed in Table 1. *E. coli* K-12 strain TG1 [Δ(*lac-proAB*) *supE thi hsdD5*/F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ* ΔM15] (9a) was used for plasmid constructions and as a host for M13 bacteriophages. Standard procedures were used to transform *Escherichia coli* (31), and selection was done on LB plates (31) supplemented with ampicillin plus chloramphenicol (50 and 2.5 μg/ml, respectively) or ampicillin plus

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TABLE 1. *B. subtilis* strains and parental plasmids used in this study

Strain or plasmid	Genotype or description[a]	Source or reference
Strains		
168	*trpC2*	Laboratory stock
BD1626	*hisH2 leuA8 metB5 comA124*::(Tn*917 cat*)	10
BD1658	*hisH2 leuA8 metB5 comP*::*cat*	47
BG4024	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ cat*)	37
BG4065	*trpC2* Δ*degQ*::*cat*	48
BG4088	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ erm*)	12
QB136	*trpC2 leuA8 degU32*(Hy)	17
QB136K1	*trpC2 leuA8 degU32*(Hy) *aphA3*	pBU126[b]→QB136
QB150	*trpC2 metC3 degQ36*(Hy)	Laboratory stock
QB151	*trpC2 metC3*	Laboratory stock
QB4238	*trpC2* Δ(*degS degU*)::*aphA3*	23
QB4249	*trpC2 degQ*::pBQ105 (*degQ'-'lacZ cat*)	pBQ105[b]→168
QB4255	*trpC2 amyE*::(*degQ'-'lacZ cat*)	23
QB4260[c]	*trpC2 amyE*::(*degQ'-'lacZ cat*) Δ(*degS degU*)::*aphA3*	23
QB4261	*trpC2 leuA8 amyE*::(*degQ'-'lacZ cat*) *degU32*(Hy)	23
QB4264	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ cat*]	pBQ109[b]→168
QB4277	*trpC2* Δ*degS aphA3*	pBU123[b]→168
QB4306	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ erm*) *degU32*(Hy) *aphA3*	QB136K1→BG4088
QB4308[c]	*trpC2 amyE*::(*degQ'-'lacZ*Δ*B cat*)	pBQ114[b]→168
QB4309[c]	*trpC2 amyE*::(*degQ'-'lacZ*Δ*A cat*)	pBQ115[b]→168
QB4310[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ*Δ*A cat*]	pBQ116[b]→168
QB4311[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ*Δ*B cat*]	pBQ117[b]→168
QB4322[c]	*trpC2 amyE*::(*degQ'-'lacZ aphA3*)	pBQ118[b]→168
QB4323[c]	*trpC2 amyE*::(*degQ'-'lacZ aphA3*) *comA124*::(Tn*917 cat*)	BD1626→QB4322
QB4327	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ erm*) *degU32*(Hy) *aphA3 comA124*::(Tn*917 cat*)	BD1626 / QB136K1 →BG4088
QB4333[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ aphA3*]	pBQ119[b]→168
QB4335[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ aphA3*] *comA124*::(Tn*917 cat*)	BD1626→QB4333
QB4339[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ cat*] Δ(*degS degU*)::*aphA3*	QB4238→QB4264
QB4341[c]	*trpC2 amyE*::(*degQ'-'lacZ*Δ*D cat*)	pBQ121[b]→168
QB4343[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ*Δ*D cat*]	pBQ123[b]→168
QB4345	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*)	BG4088→QB150
QB4346	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*) *comA124*::(Tn*917 cat*)	BD1626→QB4345
QB4347	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ cat*] *degU32*(Hy) *aphA3*	QB136K1→QB4264
QB4350	*trpC2 amyE*::(*degQ'-'lacZ cat*) Δ*degS aphA3*	QB4255→QB4277
QB4355	*trpC2 amyE*::(*sacB'-'lacZ erm*) Δ*degS aphA3*	BG4088→QB4277
QB4356	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ erm*) *comA124*::(Tn*917 cat*)	BD1626→BG4088
QB4361	*trpC2 amyE*::(*degQ'-'lacZ aphA3*) *comP*::*cat*	BD1658→QB4322
QB4374	*trpC2 amyE*::(*degQ'-'lacZ aphA3*) pBQ125	pBQ125[b]→QB4322
QB4378	*trpC2 amyE*::(*degQ'-'lacZ aphA3*) pBQ127	pBQ127[b]→QB4322
QB4382	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ cat*] Δ*degS aphA3*	pBQ109[b]→QB4277
QB4384[c]	*trpC2 amyE*::(*degQ'-'lacZ*Δ*A aphA3*)	pBQ129[b]→168
QB4385[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*A aphA3*]	pBQ130[b]→168
QB4386[c]	*trpC2 amyE*::(*degQ'-'lacZ* Δ*A aphA3*) *comA124*::(Tn*917 cat*)	BD1626→QB4384
QB4387[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*A aphA3*] *comA124*::(Tn*917 cat*)	BD1626→QB4385
QB4388[c]	*trpC2 amyE*::(*degQ'-'lacZ* Δ*A cat*) Δ(*degS degU*)::*aphA3*	QB4238→QB4309
QB4389[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*A cat*] Δ(*degS degU*)::*aphA3*	QB4238→QB4310
QB4390	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ aphA3*] *comP*::*cat*	BD1658→QB4333
QB4391	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*) *comP*::*cat*	BD1658→QB4345
QB4392	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*) Δ(*degS degU*)::*aphA3*	QB4238→QB4345
QB4393	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*) Δ*degS aphA3*	QB4277→QB4345
QB4396[c]	*trpC2 amyE*::(*degQ'-'lacZ* Δ*D cat*) Δ(*degS degU*)::*aphA3*	QB4238→QB4341
QB4397[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*D cat*] Δ(*degS degU*)::*aphA3*	QB4238→QB4343
QB4398	*trpC2 comQ*::*aphA3*	pBQ131[b]→168
QB4399	*trpC2 metC3* Δ*degQ*::*cat*	BG4065→QB151
QB4400	*trpC2 amyE*::(*degQ'-'lacZ cat*) *comQ*::*aphA3*	QB4398→QB4255
QB4402	*trpC2 hisA1 thr-5 amyE*::(*sacB'-'lacZ cat*) *comQ*::*aphA3*	QB4398→BG4024
QB4406	*trpC2 metC3 degQ36*(Hy) *amyE*::(*sacB'-'lacZ erm*) *comQ*::*aphA3*	QB4398→QB4345
QB4410[c]	*trpC2 amyE*::(*degQ'-'lacZ* Δ*B aphA3*)	pBQ134[b]→168
QB4411[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*B aphA3*]	pBQ135[b]→168
QB4412[c]	*trpC2 amyE*::(*degQ'-'lacZ* Δ*B aphA3*) *comA124*::(Tn*917 cat*)	BD1626→QB4410
QB4413[c]	*trpC2 amyE*::[*degQ36*(Hy)*'-'lacZ* Δ*B aphA3*] *comA124*::(Tn*917 cat*)	BD1626→QB4411
Plasmids		
pBQ1	3-kbp fragment[d] carrying the *degQ* gene	3
pNPRS15	1.1-kbp *Eco*RV fragment carrying the *degQ36*(Hy) mutation	48
pBU113	2.1-kbp *Eco*RI-*Eco*RV fragment carrying the *degS* gene and fusing codon 56 of *degU* to codon 8 of *lacZ*	23

[a] *cat* is the pC194 chloramphenicol acetyltransferase gene, *erm* is the Tn*917* erythromycin resistance gene, and *aphA3* is the *S. faecalis* kanamycin resistance gene. Plasmid descriptions show the *B. subtilis* chromosomal DNA insert.

[b] Described in the text. Arrows indicate construction by transformation.

[c] Used in Table 5.

[d] Obtained from a chromosomal DNA *Sau3*A1 partial digest.

kanamycin (100 and 5 μg/ml, respectively). Transformation of *B. subtilis* was done as previously described, by using plasmid or chromosomal DNA (4, 16), and selection was carried out on SP plates (3) containing chloramphenicol (5 μg/ml), kanamycin (5 μg/ml), or erythromycin plus lincomycin (1 and 25 μg/ml, respectively).

Media. *E. coli* was grown in LB broth, and *B. subtilis* was grown in Penassay antibiotic medium 3 (Difco Laboratories, Detroit, Mich.) or C minimal medium (23) supplemented with auxotrophic requirements (50 mg/liter) and the following nutrients: 2% glucose–50 mM potassium glutamate (CGE medium); 20 mM potassium succinate–50 mM potassium glutamate (CSE medium); 2% glucose–0.5% casein hydrolysate (CGCH medium); or 2% sucrose–0.2% casein hydrolysate (CScrCH medium).

Amino acid deprivation was achieved by growing cells to the mid-exponential growth phase in CGCH medium and suspending them in nitrogen-free glucose-phosphate medium as previously described (23). Phosphate limitation was achieved by transferring exponentially growing cells (optical density at 600 nm, 0.2) from glucose-amino acid (GAA) medium (2% glucose, 0.5 mM $MgSO_4$, 0.01 mM $MnSO_4$, 22 mg of ferric ammonium citrate per liter, 100 mg of each L-amino acid per liter) with 10 mM potassium phosphate buffer (pH 7.0) to GAA medium with 0.2 mM potassium phosphate and 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH adjusted to 7.0 with KOH).

Growth in the presence of decoyinine was done as follows. Cells were grown in CGE medium to an optical density at 600 nm of 0.2. The culture was then divided in two, and decoyinine (100-mg/ml solution dissolved in 1 M KOH) was added to one of the cultures to a final concentration of 250 μg/ml. Decoyinine U-7984 was a generous gift from R. M. Clarke of The Upjohn Co., Kalamazoo, Mich.

Levansucrase, α-amylase, and protease production was detected by the appropriate plate assays as previously described (3, 16, 23).

Plasmids and plasmid construction. Plasmids pBQ1, pBU113, and pNPRS15 are briefly described in Table 1. Plasmid pIS112 is a vector allowing construction of translational fusions with codon 8 of β-galactosidase (18). Plasmid pAF1 (9), a derivative of ptrpBG1 (36), carries the pC194 chloramphenicol resistance determinant *cat* and a promoterless *lacZ* gene between two fragments of the *B. subtilis amyE* gene. Derivatives of plasmids pIS112 and pAF1 cannot replicate in *B. subtilis* but can integrate into the chromosome via homologous recombination.

Plasmid pBU126, a derivative of plasmid pIS112, was constructed by insertion of a 1.5-kbp *Cla*I fragment carrying the *aphA3* kanamycin resistance determinant from *Streptococcus faecalis* (43) at the unique *Sph*I site of plasmid pBU113, upstream from *degS* and in the opposite orientation. Plasmid pBU126 was then linearized at the unique *Bst*BI site within *degS* and introduced into *degU32*(Hy) strain QB136. Km^r Cm^s integrants arose through a double-crossover event which placed the kanamycin resistance determinant upstream from *degS* in the constructed strain, QB136K1. This construction was used to introduce the *degU32*(Hy) mutation into different genetic backgrounds by using kanamycin selection.

Plasmid pBU123 was constructed from plasmid pBU126 by eliminating a 1.2-kbp *Xba*I-*Bst*BI fragment containing most of the *degS* gene. The plasmid was linearized by using the unique *Sca*I site of pIS112 and introduced into *B. subtilis* 168 by using kanamycin selection, removing nearly all of the *degS* coding sequence through a double-crossover event in the constructed strain, QB4277. The deletion was verified by polymerase chain reaction (PCR) amplification and DNA sequencing.

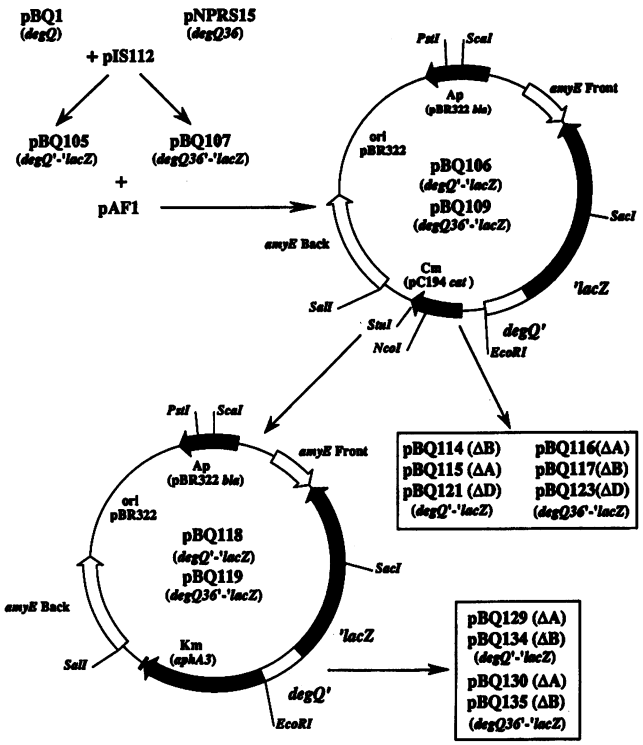

FIG. 1. Structures of plasmids used in this study, carrying *degQ'-'lacZ* translational fusions and allowing direct selection of single-copy integration at the *B. subtilis amyE* locus by either chloramphenicol or kanamycin selection. Details of plasmid constructions are described in Materials and Methods.

Plasmid pBQ105 was constructed by cloning a 569-bp *Eco*RV-*Bcl*I fragment from plasmid pBQ1 (3), carrying the first 33 codons of *degQ*, between the *Sma*I and *Bam*HI sites of pIS112, thus fusing codon 33 of *degQ* to codon 8 of *lacZ*. Introduction of plasmid pBQ105 into *B. subtilis* 168 produced strain QB4249, carrying the *degQ'-'lacZ* fusion at the *degQ* locus, integrated through a Campbell-type recombination event.

Plasmid pBQ107 was constructed in the same way as plasmid pBQ105, by using the corresponding *Eco*RV-*Bcl*I fragment from plasmid pNPRS15 (48), carrying the *degQ36* (Hy) mutation, thus placing the *degQ36* modified promoter upstream from the *degQ'-'lacZ* translational gene fusion. The fusions were then transferred to plasmid pAF1 by using unique *Eco*RI and *Sac*I restriction sites located, respectively, upstream of and within the *lacZ* gene. Plasmids pBQ106 and pBQ109 are the corresponding pAF1 derivatives of plasmids pBQ105 and pBQ107, respectively, and their general structures are shown in Fig. 1. Plasmids pBQ106 and pBQ109 were linearized and introduced into *B. subtilis* 168 to give strains QB4255 and QB4264, respectively.

Plasmids pBQ118 and pBQ119 are kanamycin resistance-determining derivatives of plasmids pBQ106 and pBQ109, respectively, and were constructed as follows. The *aphA3* kanamycin resistance determinant was inserted at the *Stu*I restriction site of the *cat* gene, followed by deletion of the

cat coding sequence between the *Eco*RI site upstream from the *cat* gene and the *Nco*I site within the coding sequence. The general structures of these plasmids are shown in Fig. 1.

Constructed plasmids were linearized by using unique *Pst*I or *Sca*I sites and introduced into *B. subtilis* 168 by chloramphenicol or kanamycin selection, allowing integration into the chromosome by homologous recombination at the *amyE* locus through a double-crossover event and disruption of the *amyE* gene by the translational gene fusion (α-amylase deficiency phenotype).

PCRs were used to introduce *Eco*RI restriction sites at various positions upstream from *degQ*. The *Eco*RI-*Bcl*I fragments generated in this way were cloned between the *Eco*RI and *Bam*HI sites of pIS112, creating translational fusions between codon 33 of *degQ* and codon 8 of *lacZ*. The fusions were then transferred to the pAF1 plasmid vector as described above, creating plasmids deleted for different regions upstream from the *degQ'-'lacZ* fusion. Plasmids pBQ114 (Δ*B*), pBQ115 (Δ*A*), and pBQ121 (Δ*D*) are deleted derivatives of plasmid pBQ106 constructed in this way, and plasmids pBQ116 (Δ*A*), pBQ117 (Δ*B*), and pBQ123 (Δ*D*) are deleted derivatives of plasmid pBQ109 (for the positions of the corresponding deletions, see Fig. 5 and Table 5; plasmid constructions are summarized in Fig. 1). Plasmids pBQ129 (Δ*A*) and pBQ134 (Δ*B*) are deleted derivatives of plasmid pBQ118, and plasmids pBQ130 (Δ*A*) and pBQ135 (Δ*B*) are deleted derivatives of plasmid pBQ119 (Fig. 1).

Plasmids pBQ125 and pBQ127 were constructed by cloning *Eco*RI-*Sma*I PCR-generated fragments carrying regions upstream from *degQ* and the *degQ* promoter region, respectively, between the *Eco*RI and *Sma*I sites of the pMK4 shuttle plasmid vector (41), which replicates in both *E. coli* and *B. subtilis*.

Plasmid pBQ131 was constructed by inserting the *aphA3* kanamycin resistance determinant at the unique *Sna*BI site of pBQ1.

DNA manipulations. Standard procedures were used to extract plasmids from *E. coli* (3, 31). Restriction enzymes, T4 DNA polymerase, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were used as recommended by the manufacturers. When necessary, 5′ and 3′ protruding ends were repaired to flush ends by using Klenow DNA polymerase, T4 DNA polymerase, and deoxyribonucleoside triphosphates. DNA fragments were purified from agarose gels by using Geneclean or Mermaid kits (Bio 101, La Jolla, Calif.).

DNA sequences were determined by using the dideoxy-chain termination method (32) and modified T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, Ohio). Templates used for DNA sequencing were either single-stranded M13 phages, double-stranded plasmid minipreparations (15), or single-stranded PCR products produced through asymmetric amplification (13, 38).

Chromosomal DNA was isolated from exponentially growing *B. subtilis* cells as previously described (23). Oligonucleotide primers were synthesized by the β-cyanoethyl phosphoramidite method by using a Milligen/Biosearch Cyclone Plus synthesizer (Millipore, Inc., Burlington, Mass.) and used for amplification and sequencing reactions without purification.

PCRs (24, 30) were done by using *Thermus aquaticus* DNA polymerase as recommended by New England BioLabs, Inc., Beverly, Mass. Oligonucleotide primers used for PCRs included mismatches allowing creation of *Eco*RI or *Sma*I restriction sites. After an initial denaturation step of 10 min at 95°C, amplification was done for 25 rounds. The DNA was denatured at 95°C for 1 min, annealed at 55°C for 1 min, and extended at 72°C for 2 min. Samples were successively extracted with phenol and chloroform, ethanol precipitated, and digested with appropriate restriction enzymes before gel purification.

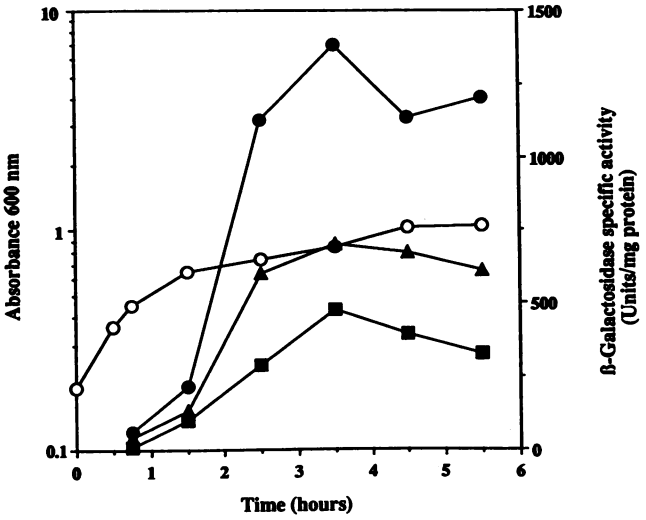

FIG. 2. Time course of *degQ* expression as measured by a *degQ'-'lacZ* translational fusion under conditions of phosphate starvation. Exponentially growing cells (optical density at 600 nm, 0.2) were transferred from GAA medium with 10 mM phosphate to GAA medium with 0.2 mM phosphate. β-Galactosidase specific activities were determined as a function of time after transfer to GAA medium with 0.2 mM phosphate. Symbols: ●, strain QB4255; ▲, strain QB4260 (Δ*degS degU*); ■, strain QB4261 [*degU32*(Hy)]; ○, A_{600} (the growth curve represents an average value for the three strains).

β-Galactosidase assays. *B. subtilis* cells containing *lacZ* fusions were grown in the indicated media. β-Galactosidase specific activities were determined as previously described and expressed as Miller units per milligram of protein (20, 23). The values indicated represent averages from at least three independent assays.

B. subtilis colonies expressing *lacZ* fusions were detected by overlaying colonies with 8 ml of soft agar (7.5 mg/ml) containing lysozyme (2 mg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (100 μg/ml).

Nucleotide sequence accession number. The nucleotide sequence data reported here have been submitted to GenBank and assigned accession no. M60044.

RESULTS

Expression of the *degQ* gene under different nutritional conditions. Previous results have shown that *degQ* gene expression is very low in cells growing exponentially in the presence of glucose (23, 48). During this growth phase, carbon, nitrogen, and phosphate sources are in excess and therefore do not limit the growth rate. Two sets of growth-limiting conditions, amino acid deprivation and poor carbon sources, led to a strong increase in *degQ* gene expression (23).

A third set of conditions, phosphate starvation, also led to an increased rate of DegQ synthesis (Fig. 2). Strain QB4255 carries a single chromosomal copy of a *degQ'-'lacZ* translational fusion (see Materials and Methods). Cells growing exponentially in GAA medium with 10 mM phosphate were

TABLE 2. Effects of *degS* and *degU* upon *degQ'-'lacZ* expression under different growth conditions[a]

Growth conditions	β-Galactosidase sp act (U/mg of protein)							
	degQ'-'lacZ[b]				*degQ36*(Hy)*'-'lacZ*[b]			
	QB4255	QB4260 (Δ*degS degU*)	QB4261 [*degU32*(Hy)]	QB4350 (Δ*degS*)	QB4264	QB4339 (Δ*degS degU*)	QB4347 [*degU32*(Hy)]	QB4382 (Δ*degS*)
1. Glucose as the carbon source (CGE medium)	140	35	20	100	6,970	865	210	1,960
2. Poor carbon sources (CSE medium)	875	175	430	240	12,900	4,040	7,230	4,830
3. Excess phosphate and amino acids (GAA medium with 10 mM phosphate)	55	40	8	35	1,090[c]	860	320	1,670
4. Phosphate starvation (GAA medium with 0.2 mM phosphate)	1,390	705	480	715	14,700	5,780	1,000	5,800
5. Amino acid deprivation (glucose-phosphate medium)	1,210	100	30	500	23,600[c]	7,110	540	13,400

[a] β-Galactosidase specific activities represent measurements made during the exponential growth phase (conditions 1 to 3) or maximum expression levels (conditions 4 and 5).
[b] Relevant genotypes are included with strain names.
[c] Determined for strain QB4333, which carries an identical *degQ36*(Hy)*'-'lacZ* fusion (Table 1).

transferred to GAA medium with a growth-limiting phosphate concentration (0.2 mM), and β-galactosidase specific activities were determined as a function of time (Fig. 2). As soon as the cells entered the stationary phase because of phosphate starvation, *degQ'-'lacZ* expression strongly increased (strain QB4255; Fig. 2 and Table 2).

It was previously shown that the *degQ36*(Hy) mutation, a single base change at position −10, resulted in overexpression of *degQ* (48). Although the *degQ36* mutation greatly increased *degQ* gene expression, it had little or no effect on regulation by nutrient depletion. QB4264 and QB4333 are chloramphenicol- and kanamycin-resistant strains, respectively, carrying identical *degQ'-'lacZ* fusions expressed from the *degQ36*(Hy) promoter (see Materials and Methods). This expression was increased under conditions of phosphate starvation (Fig. 3, strain QB4333), growth with poor carbon sources, or amino acid deprivation (Table 2, strain QB4264).

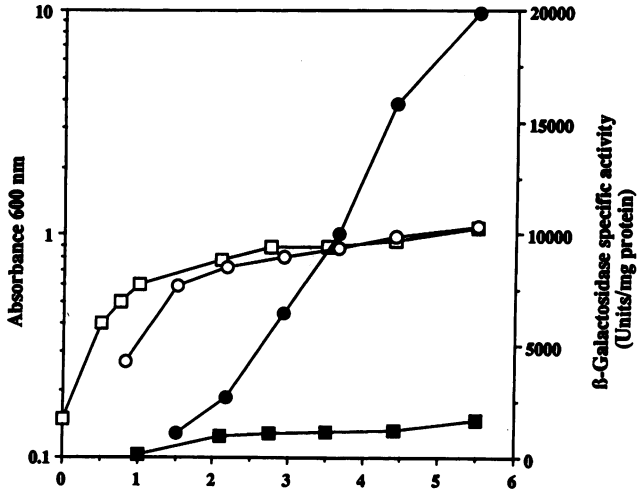

FIG. 3. Time course of *degQ* expression driven by the *degQ36* (Hy) promoter, as measured by a *degQ36*(Hy)*'-'lacZ* translational fusion, under conditions of phosphate starvation. The time scale and assay conditions are as indicated in the legend to Fig. 2. β-Galactosidase activity symbols: ●, strain QB4333; ■, strain QB4335 (*comA124*). A_{600} symbols: ○, strain QB4333; □, strain QB4335 (*comA124*).

Control experiments indicated that *degQ* expression increased specifically in response to amino acid deprivation rather than nitrogen starvation, since no increase was seen under conditions of nitrogen starvation after cells were grown with ammonium sulfate as the only nitrogen source (data not shown).

Comparing the levels of expression in strains QB4255 and QB4264, it was obvious that the *degQ36* modified promoter led to a 10- to 50-fold increase in *degQ* expression under the various conditions tested (Table 2).

The *degQ36* promoter turned out to be highly efficient in *B. subtilis*. Its efficiency was compared with that of the promoter of the *sacB* gene encoding levansucrase. The rate of sucrose-induced β-galactosidase synthesis driven by the *sacB* promoter was determined in strain QB4306, which contains a *sacB'-'lacZ* fusion and a *degU32*(Hy) mutation. The value obtained, approximately 7,000 U/mg of protein, was lower than the maximum rate of *degQ36*-driven β-galactosidase synthesis (approximately 23,000 U/mg of protein [Table 2]). Keeping in mind that levansucrase production in a *degU32*(Hy) strain represents about 8% of the total protein synthesis (7), it is possible that the *degQ36* promoter allows an even higher rate of synthesis.

Limitations of carbon, nitrogen, and phosphate sources correspond to conditions which signal initiation of sporulation (21, 33). Expression of *degQ* may therefore be regulated in a way similar to that of early sporulation genes.

Effect of decoyinine on the level of *degQ* expression. It has previously been shown that sporulation of *B. subtilis* can be initiated by addition of decoyinine, a specific inhibitor of GMP synthetase, to the culture medium (19, 21). We therefore examined whether decoyinine affected the level of expression of *degQ*. Cells were grown in CGE medium, and β-galactosidase specific activities were determined as a function of time with or without decoyinine (250 μg/ml) in the culture medium (Fig. 4). Expression of *degQ'-'lacZ* in strain QB4255 was clearly stimulated by decoyinine, overriding repression by glucose. Expression of *degQ'-'lacZ* from the *degQ36* promoter also increased approximately eightfold in the presence of decoyinine (data not shown).

Control of *degQ* gene expression by the DegS-DegU regulatory pair. We postulated earlier that the DegS-DegU two-component system affects *degQ* expression. This was deduced from comparison of the rates of β-galactosidase synthesis in three *degQ'-'lacZ* strains: QB4255, carrying

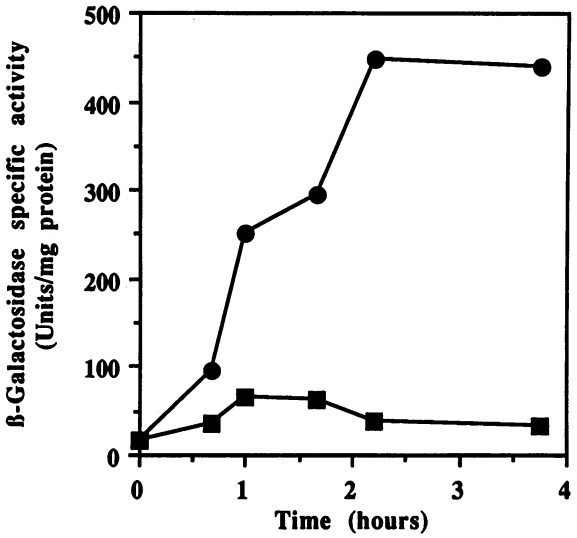

FIG. 4. Expression of *degQ'-'lacZ* in strain QB4255 in the presence or absence of decoyinine. Cells were grown to an optical density at 600 nm of 0.2 in CGE medium. The culture was then divided in two and decoyinine was added to one of the cultures. β-Galactosidase specific activities were determined as a function of time after addition of decoyinine. Symbols: ●, with decoyinine (250 μg/ml); ■, without decoyinine.

wild-type copies of *degS* and *degU*; QB4260, from which *degS* and *degU* were deleted; and QB4261, carrying a *degU32*(Hy) mutation (23). Strains QB4260 and QB4261 both displayed lower rates of synthesis of β-galactosidase than strain QB4255 under conditions of amino acid deprivation or growth with poor carbon sources (23; Table 2).

This was also true under conditions of phosphate limitation (Fig. 2 and Table 2). Expression of *degQ'-'lacZ* from both the wild-type promoter and the *degQ36*(Hy) promoter was lowered in strains deleted for *degS* and *degU* or carrying a *degU32*(Hy) mutation under the various conditions tested (Table 2). This may suggest that the *degU32*(Hy) mutation modifies the DegU protein in such a way that it no longer acts as a positive regulator of *degQ* gene expression.

Previous results have shown that two sets of target genes are regulated in a distinct manner by the DegS-DegU two-component system: genes encoding degradative enzymes require two functional regulatory proteins (DegS and DegU) for their expression, while the presence of a functional DegU protein alone is sufficient for expression of competence (23, 28). We therefore examined whether DegU alone or both DegS and DegU control *degQ* gene expression.

We first constructed strain QB4277, from which most of the *degS* sequence was deleted (see Materials and Methods), allowing full expression of *degU* from the *degS-degU* operon promoter, upstream from *degS*, as previously shown (23). As mentioned above, competence in strain QB4277 was only slightly reduced (Table 3). The *degQ'-'lacZ* translational fusion was then introduced into strain QB4277 to give strain QB4350. Expression of *degQ'-'lacZ* in CSE medium was decreased approximately three- to fourfold in this strain (Table 2), indicating that the presence of a functional DegS protein contributes to expression of *degQ*. A similar effect was observed for *degQ36*-driven expression of *degQ'-'lacZ*, which was lowered in strain QB4382, carrying the *degS* deletion, under the different conditions tested (Table 2). However, the effect of deletion of *degS* or both *degS* and *degU* was minor under conditions of phosphate starvation, since *degQ* expression was lowered only twofold.

TABLE 3. Effects of *degS* and *degQ* upon transformation frequency

Strain	Genotype	Transformation frequency[a]
168	*trpC2*	1
QB4277	*trpC2 ΔdegS aphA3*	0.25
QB151	*trpC2 metC3*	1
QB150	*trpC2 metC3 degQ36*(Hy)	0.13
QB4399	*trpC2 metC3 ΔdegQ::cat*	0.78

[a] Transformation frequencies are expressed relative to those of the corresponding isogenic strains (168 and QB151) and were determined by using either plasmid or chromosomal DNA at 2 μg/ml with selection for chloramphenicol or erythromycin resistance, respectively.

Expression of *degQ* in strains carrying the *degU32*(Hy) mutation or deletion of *degS* or both *degS* and *degU* showed some variation under the different conditions used (Table 2). However, *degQ* gene expression in these strains was still increased under conditions of amino acid deprivation, phosphate depletion, or growth with poor carbon sources. This suggests that *degS* and *degU* are not significantly involved in the nutritional regulation of *degQ* gene expression under the conditions examined.

Regulation of *degQ* expression by ComP-ComA. In addition to DegS-DegU, a second two-component system, ComP-ComA, controls the expression of genetic competence in *B. subtilis* (46, 47). The *comP* and *comA* genes are located on the *B. subtilis* chromosome downstream from *degQ* (47). To examine whether *degQ* gene expression is also controlled by the ComP-ComA regulatory proteins, the disrupted *comA124* gene was introduced into strain QB4322, which carries a single chromosomal copy of a *degQ'-'lacZ* fusion, to give strain QB4323. Expression of *degQ'-'lacZ* was strongly dependent upon ComA, since it was decreased approximately 50- to 100-fold in strain QB4323, carrying the *comA124* disruption, under the different conditions examined (Table 4). Expression of *degQ'-'lacZ* driven by the *degQ36* promoter was also strongly decreased when *comA* was disrupted (strain QB4335), indicating that expression driven by the modified promoter is still dependent upon the presence of a functional *comA* gene (Fig. 3 and Table 4).

Expression of *degQ* when *comA* was disrupted was still repressed by glucose and increased under conditions of phosphate depletion or growth with poor carbon sources. However, the increase in *degQ* gene expression under conditions of amino acid deprivation was not as strong in a strain carrying a *comA124* disruption (Table 4, conditions 3 and 5, strains QB4333 and QB4335). This suggests that regulation of *degQ* gene expression by amino acid deprivation is ComA dependent. On the other hand, ComA does not appear to be involved in regulation of *degQ* gene expression by catabolite repression or phosphate depletion (Table 4).

A *comP* disruption, allowing *comA* expression from a promoter between *comP* and *comA* (47), was introduced into strain QB4322, which carries a *degQ'-'lacZ* fusion, to give strain QB4361. Expression of *degQ'-'lacZ* during growth with poor carbon sources (CSE medium) was diminished approximately 20-fold, from 890 U/mg of protein (strain QB4322) to 45 U/mg of protein (strain QB4361). Expression of *degQ'-'lacZ* from the *degQ36* promoter was lowered approximately 10-fold in CSE medium from 13,300 U/mg of protein (strain QB4333) to 1,440 U/mg of protein in strain

TABLE 4. Effect of *comA* upon *degQ'-'lacZ* expression under different growth conditions[a]

Growth conditions	β-Galactosidase sp act (U/mg of protein)			
	degQ'-'lacZ[b]		*degQ36*(Hy)*'-'lacZ*[b]	
	QB4322	QB4323 (*comA124*)	QB4333	QB4335 (*comA124*)
1. Glucose as the carbon source (CGE medium)	140[c]	2	6,700	235
2. Poor carbon sources (CSE medium)	890	17	13,300	560
3. Excess phosphate and amino acids (GAA medium with 10 mM phosphate)	55[c]	3	1,090	95
4. Phosphate starvation (GAA medium with 0.2 mM phosphate)	1,350	14	17,700	1,680
5. Amino acid deprivation (glucose-phosphate medium)	1,210[c]	ND[d]	23,600	285

[a] β-Galactosidase specific activities represent measurements made during the exponential growth phase (conditions 1 to 3) or maximum expression levels (conditions 4 and 5).
[b] Relevant genotypes are included with strain names.
[c] Determined for strain QB4255, which carries an identical *degQ'-'lacZ* fusion (Table 1).
[d] ND, Not determined.

QB4390, which carries a *comP* disruption. This indicates that both ComP and ComA regulate *degQ* gene expression.

The ComP-ComA system also controls expression of the late competence genes *comC* and *comG*. Expression of *comC* and *comG* is stimulated by nitrogen limitation in the presence of glucose and repressed by glutamine (2, 10, 28, 47). It has been suggested that the ComP-ComA system is involved in this metabolic control (47). Like late competence genes, *degQ* is controlled by ComP-ComA. However, *degQ* is expressed at a very low level in the presence of glucose, whereas late competence genes are not subject to catabolite repression by glucose (2) (see Discussion).

Deletion of the *degQ* gene did not cause a detectable decrease in competence under the experimental conditions used (Table 3, strain QB4399). The high level of DegQ synthesis in a *degQ36*(Hy) mutant was accompanied by a slightly decreased level of competence (Table 3, strain QB150).

Nucleotide sequence of the *degQ* region. Sequence data published for the *degQ* gene extended to position −99 with respect to the transcriptional start site (48). To carry out a preliminary characterization of regulatory regions upstream from *degQ*, further nucleotide sequence data were necessary. A 1,185-bp *Eco*RV fragment containing *degQ* was isolated from plasmid pBQ1 and transferred to M13mp18. The nucleotide sequence of the entire *Eco*RV fragment was determined, extending from −393 upstream from *degQ* to position +789 with respect to the *degQ* transcription initiation site. The nucleotide sequence upstream from *degQ* shown in Fig. 5 partially overlapped previously published sequence data (46).

Is *comQ* a novel regulatory gene controlling competence and *degQ* gene expression? Sequence analysis of the region directly downstream from *degQ* revealed the first 130 codons of an open reading frame. This open reading frame was disrupted by insertion of the *aphA3* kanamycin resistance determinant at a unique *Sna*BI site within the open reading frame in plasmid pBQ1 to give plasmid pBQ131. Introduction of the linearized plasmid into *B. subtilis* 168 resulted in strain QB4398, which had a very low transformation frequency, diminished more than 1,000-fold compared with that of the wild-type strain (data not shown). This suggests that this open reading frame, designated *comQ*, encodes a product that is essential to the development of genetic competence. An alternative possibility is that the *comQ* disruption has a polar effect upon expression of the *comP* and *comA* genes, located immediately downstream from *comQ*. Recent results, however, suggest that this is not the case and that *comQ* is directly involved in regulating the expression of competence genes (47a).

The *comQ* disruption was introduced into strain QB4255, carrying a *degQ'-'lacZ* fusion, to give strain QB4400. Expression of *degQ'-'lacZ* during growth with poor carbon sources (CSE medium) was reduced 24-fold, from 890 U/mg of protein (strain QB4255) to 37 U/mg of protein (strain QB4400), suggesting that *comQ* also controls *degQ* expression. A similar decrease in *degQ'-'lacZ* expression was seen when cells carrying a *comQ* disruption were grown in the presence of glucose (CGE medium) (data not shown).

The nucleotide sequence and characterization of the *comQ* gene will be described elsewhere.

Control regions upstream from the *degQ* gene. As described above, *degQ* gene expression appears to be affected by four distinct regulatory systems: DegS-DegU, ComP-ComA, catabolite repression, and regulation by phosphate. We tried to locate the targets of these regulatory systems upstream from the *degQ* gene. We used plasmids carrying *degQ'-'lacZ* fusions in which β-galactosidase expression is driven by either the wild-type promoter (plasmid pBQ106) or the *degQ36*(Hy) modified promoter (plasmid pBQ109). A series of deleted derivatives were then constructed from these

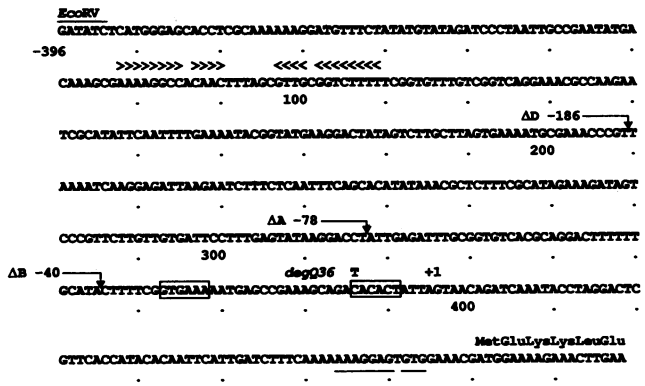

FIG. 5. Nucleotide sequence of the *degQ* upstream region. Deletion endpoints are indicated by vertical arrows and numbered with respect to the transcription start site (+1). −35 and −10 sequences are boxed, the *degQ36* mutation is indicated, and the Shine-Dalgarno sequence is underlined. The opposing arrowheads indicate a palindromic structure upstream from the *degQ* gene.

TABLE 5. Effects of upstream deletions on expression of *degQ'-'lacZ* in different genetic backgrounds[a]

Deletion	Deletion endpoint	β-Galactosidase sp act (U/mg of protein)[b]					
		degQ'-'lacZ			*degQ36*(Hy)'-'*lacZ*		
		Wild type	*comA124*	Δ*degS degU*	Wild type	*comA124*	Δ*degS degU*
	−393	890	17	177	13,200	560	3,890
Δ*D*	−186	124	ND[c]	124	3,270	ND	3,680
Δ*A*	−78	138	19	130	3,620	430	3,750
Δ*B*	−40	22	23	ND	248	245	ND

[a] The strains used are indicated in Table 1.
[b] β-Galactosidase specific activities were determined in extracts prepared from cells growing exponentially in CSE medium.
[c] ND, Not determined.

plasmids. The DNA fragments upstream from *degQ'-'lacZ* in the parental plasmids were replaced by DNA fragments from which part of the upstream region was missing. These fragments were synthesized in PCRs, allowing us to define the endpoints of the deletions and to introduce appropriate restriction sites at these endpoints. The DNA fragments containing deleted derivatives of the *degQ'-'lacZ* fusions were then introduced as single copies at the *amyE* locus (see Materials and Methods). Deletion endpoints are indicated in Fig. 5.

We established first that all of these target sites were located downstream from −393. The upstream region to position −393 was indeed sufficient for full expression of *degQ* during growth with poor carbon sources (CSE medium), since the levels of *degQ'-'lacZ* expression (860 U/mg of protein) were identical in strain QB4255, which carries the fusion at the *amyE* locus, and strain QB4249, which carries the same fusion at the *degQ* locus, integrated through a Campbell-type recombination event (see Materials and Methods).

We reasoned that the *degQ* control regions could contain direct or indirect ComA and DegU target sites upstream from the promoter, since regulation by two-component systems often involves targets located upstream from the promoter regions (11, 40). We showed that *degQ'-'lacZ* fusions with upstream sequences extending to position −78 had the same expression levels in CSE medium as the −186 deletion, remaining approximately four- to sevenfold lower than those of fusions with 393 bp upstream from the transcription start site (Table 5). Regions between −186 and −393 seemed to be involved in positive regulation responsible for a four- to sevenfold increase in expression. This increase in expression appeared to be due to the DegS-DegU two-component system. Indeed, deletion of *degS* and *degU* decreased the level of expression of a *degQ'-'lacZ* fusion approximately four- to fivefold when upstream sequences were present to position −393. However, such a deletion of *degS* and *degU* had no effect on expression of *degQ'-'lacZ* fusions when upstream regions were deleted to position −78 or −186 (Table 5). We concluded that a DegS-DegU target was located between positions −393 and −186.

It seemed likely that a ComA target site was located downstream from −78, since a deletion to −40 showed a sharp cutoff in the rate of β-galactosidase synthesis whereas deletion to −78 retained a relatively high level of expression (Table 5). To test this hypothesis, we introduced the *comA124* disruption into strains which carry −78 deletions and either the wild-type promoter or the *degQ36* promoter upstream from the *degQ'-'lacZ* fusion. The *comA124* derivatives synthesized β-galactosidase at lower levels than their parental strains, which carry a functional *comA* gene (Table 5). It appears, therefore, that sequences necessary for activation of *degQ* expression by ComA are still present when upstream regions are deleted to position −78. Introducing the *comA124* disruption into strains carrying deletions to −40 had no effect on *degQ'-'lacZ* expression (Table 5), suggesting that the *comA* target site is no longer present. Expression of *degQ'-'lacZ* in strains carrying fusions deleted to −78 was strongly increased under conditions of amino acid deprivation. As mentioned above, this increase in expression was found to be *comA* dependent, as it was not as strong in a *comA124* background (Table 4, conditions 3 and 5; Table 6, conditions 4 and 6). This suggests that amino acid

TABLE 6. Effects of upstream deletions upon *degQ36*(Hy)'-'*lacZ* expression under different growth conditions[a]

Growth conditions	β-Galactosidase sp act (U/mg of protein)[b]		
	QB4385 (*degQ36'-'lacZ* Δ*A*)	QB4387 (*degQ36'-'lacZ* Δ*A* comA124)	QB4311 (*degQ36'-'lacZ* Δ*B*)
1. Glucose as the carbon source (CGE medium)	2,020	95	60
2. Glucose as the carbon source (CGE medium) with 250 μg of decoyinine per ml	ND[c]	ND	345
3. Poor carbon sources (CSE medium)	3,620	430	250
4. Excess phosphate and amino acids (GAA medium with 10 mM phosphate)	235	45	60
5. Phosphate starvation (GAA medium with 0.2 mM phosphate)	5,080	610	270
6. Amino acid deprivation (glucose-phosphate medium)	9,160	265	ND

[a] β-Galactosidase specific activities represent measurements made during the exponential growth phase (conditions 1, 3, and 4) or maximum expression levels (conditions 2, 5, and 6).
[b] Relevant genotypes are included with strain names.
[c] ND, Not determined.

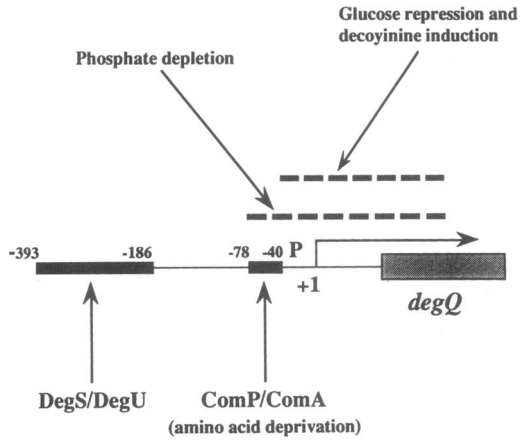

FIG. 6. Schematic diagram indicating direct or indirect regulatory targets for the DegS-DegU and ComP-ComA modulator-effector pairs (black boxes numbered with respect to the transcription start site [+1]) upstream from the *degQ* gene. P indicates the *degQ* promoter, and the direction of transcription is shown by the horizontal arrow.

deprivation is a signal triggering ComP-ComA-mediated control of *degQ* gene expression.

Phosphate starvation led to increases in the levels of expression of about 20-fold and 4-fold in strains QB4385 and QB4311, containing upstream sequences extending to −78 and −40, respectively (Table 6). The target for regulation by phosphate is therefore located downstream from −78. A similar increase in the level of expression due to phosphate depletion was observed in a strain carrying a *comA* disruption (Table 6), suggesting that phosphate regulation was distinct from ComP-ComA-mediated regulation.

Expression of *degQ'-'lacZ* fusions with upstream regions extending to −40 was still repressed approximately fourfold by glucose and remained fully inducible by decoyinine (Table 6). The target which is probably involved in both catabolite repression and decoyinine induction thus appears to be located downstream from −40.

These results indicate that four targets could be distinguished upstream from the *degQ* gene (Fig. 6). (i) A target for the DegS-DegU two-component system is located between positions −393 and −186 with respect to the transcription initiation site. (ii) A ComA target is located between −78 and −40. (iii) A target allowing regulation of *degQ* gene expression as a function of the phosphate concentration appears to be located downstream from −78. (iv) A target which is probably involved in both catabolite repression and decoyinine induction is located downstream from −40.

Expression of *comC* and *comG*, two of the late competence genes controlled by *comA*, has been shown to be lowered in strains carrying the *comC* or *comG* promoter on multicopy plasmids (1, 22). It was suggested that this was due to titration of a competence-specific transcription factor present in limiting amounts (22). To examine whether a comparable effect could be seen for *degQ* expression, plasmid pBQ125, carrying regions −186 to −60 with respect to the *degQ* transcription start site, was constructed (see Materials and Methods) and introduced into strain QB4322 to give strain QB4374. The level of *degQ* gene expression was measured during growth with poor carbon sources (CSE medium). The expression level of the chromosomal *degQ'-'lacZ* fusion under these conditions was lowered from 850 U/mg of protein (strain QB4322) to 250 U/mg of protein (strain QB4374). This three- to fourfold decrease suggests that the regions from −186 to −60 upstream from the *degQ* promoter are sufficient for titration of a positive regulator of *degQ* expression. Candidates for such a regulatory gene could be either the *comA* gene, a gene controlled by *comA*, or some unidentified gene. A similar three- to fourfold decrease in *degQ'-'lacZ* expression was seen when plasmid pBQ127, carrying the promoter region (−186 to +32), was introduced into strain QB4322 to give strain QB4378.

Expression of *sacB* in different genetic backgrounds. The results presented above demonstrated that the ComP-ComA two-component system controls *degQ* expression. Since the *degQ* gene, in turn, controls the expression of degradative enzymes, one would expect that at least under some conditions, expression of these enzymes would also be affected by the ComP-ComA system. Indeed, the five- to sixfold increase of *sacB'-'lacZ* expression in a *degQ36*(Hy) strain (Table 7, strains BG4088 and QB4345) was lost when *comA*, *comP*, or *comQ* was disrupted (Table 7, strains QB4346, QB4391, and QB4406, respectively). However, *comA* and *comQ* disruptions had no effect on *sacB'-'lacZ* expression in the presence of a wild-type *degQ* allele (Table 7, strains QB4356 and QB4402, respectively). This does not contradict the results mentioned above, since the wild-type *degQ* gene was expressed at a low level under our assay conditions: *degQ'-'lacZ* expression from the wild-type promoter in sucrose minimal medium (CScrCH) was approximately 28 U/mg of protein (strain QB4322), whereas expression from

TABLE 7. Expression of *sacB'-'lacZ* in different genetic backgrounds

Strain	Relevant genotype	β-Galactosidase sp act (U/mg of protein)[a]
BG4088	*amyE*::*sacB'-'lacZ*	39
QB4355	*amyE*::*sacB'-'lacZ* Δ*degS aphA3*	9
QB4356	*amyE*::*sacB'-'lacZ comA124*::(Tn*917 cat*)	39
QB4402	*amyE*::*sacB'-'lacZ comQ*::*aphA3*	35
QB4306	*amyE*::*sacB'-'lacZ degU32*(Hy) *aphA3*	7,360
QB4327	*amyE*::*sacB'-'lacZ degU32*(Hy) *aphA3 comA124*::(Tn*917 cat*)	8,700
QB4345	*amyE*::*sacB'-'lacZ degQ36*(Hy)	220
QB4346	*amyE*::*sacB'-'lacZ degQ36*(Hy) *comA124*::(Tn*917 cat*)	38
QB4391	*amyE*::*sacB'-'lacZ degQ36*(Hy) *comP*::*cat*	56
QB4406	*amyE*::*sacB'-'lacZ degQ36*(Hy) *comQ*::*aphA3*	72
QB4393	*amyE*::*sacB'-'lacZ degQ36*(Hy) Δ*degS aphA3*	11
QB4392	*amyE*::*sacB'-'lacZ degQ36*(Hy) Δ(*degS degU*)::*aphA3*	5

[a] β-Galactosidase specific activities were determined in extracts prepared from cells growing exponentially in CScrCH medium.

the *degQ36*(Hy) modified promoter was approximately 2,200 U/mg of protein (strain QB4264).

Both *degS* and *degU* are required for degradative enzyme synthesis. Deletion of *degS* diminished *sacB'-'lacZ* expression approximately four- to fivefold (Table 7, strain QB4355). In addition, deletion of either *degS* or both *degS* and *degU* strongly decreased *sacB'-'lacZ* expression in *degQ36*(Hy) strains (Table 7, strains QB4345, QB4393, and QB4392). However, the ComP-ComA regulatory proteins do not seem to act upon either *degS-degU* or degradative enzyme synthesis directly, since a *comA* disruption had no effect on *sacB'-'lacZ* expression in strains carrying either a wild-type *degU* allele or the *degU32*(Hy) mutation (Table 7, strains BG4088, QB4356, QB4306, and QB4327).

DISCUSSION

Several regulatory genes encoding small polypeptides of 46 to 65 amino acid residues which cause a higher level of expression of a class of extracellular proteins in *B. subtilis* have been reported, i.e., *senS* (45), *degR* (25, 42, 49), and *degQ* (3, 48). The pleiotropic effects of these genes suggest that they are part of a global control mechanism affecting the synthesis of degradative enzymes.

It was shown in this study that both the DegS-DegU and ComP-ComA two-component systems affect expression of the *degQ* regulatory gene. Expression of *degQ* was examined in this study by using a *degQ'-'lacZ* translational fusion. Although we favor the hypothesis that *degQ* gene expression is regulated transcriptionally, we cannot rule out the possibility of posttranscriptional control. Disruption of the *comA* gene decreased *degQ* gene expression about 50-fold, while deletion of the *degS* and *degU* genes decreased *degQ* gene expression about fourfold (23; this report). Deletions of the *comP* and *degS* genes, encoding protein kinases, also showed strong effects on *degQ* gene expression. Deletion of *degS*, in which *degU* gene expression was initiated at the *degS-degU* operon promoter, led to two- to fourfold decreases of *degQ* gene expression under the different conditions tested. Deletion of *comP*, still allowing expression of *comA* from a promoter located between *comP* and *comA* (47), decreased *degQ* gene expression about 20-fold.

The *degQ36* mutation, which was previously characterized (3, 48), corresponds to a promoter up mutation which strongly increases expression of the *degQ* gene but has no detectable effect on regulation by nutrient depletion. Expression from the *degQ36* promoter was still activated by the *comA* gene. The *degQ36* promoter is one of the strongest promoters which have been identified in *B. subtilis*.

We performed a deletion analysis of the DNA sequence upstream from *degQ* to identify the targets for regulation by the *comA* and *degU* genes. Targets for activation by *degU* and *comA* were located, with respect to the transcription start site, between positions −393 and −186 and between positions −78 and −40, respectively. The region between positions −393 and −186 contains a palindromic structure which may be the transcription terminator of a gene located upstream (46) or a structure involved in regulation of *degQ* gene expression.

We do not know whether the ComA and DegU effectors bind directly to the sequences upstream of the *degQ* gene or whether they act indirectly, through other regulatory genes which could then bind to the *degQ* upstream sequences. Several other genes are known to be involved in the regulation of competence genes, for example, *comL* and *csh-293* (which are thought to be part of the *srfA* operon), *mecA*, *mecB*, and *comK* (8, 14, 26–28, 44). Some of these regulatory genes seem to be involved as intermediates in the expression of late competence genes, since this expression could be restored by *mecA* or *mecB* mutations in strains carrying *degU* or *comA* disruptions (28).

Weinrauch et al. (47) proposed that the ComP-ComA system is involved in regulation of the expression of late competence genes, which is stimulated in the presence of glucose by nitrogen limitation and is, on the other hand, repressed by glutamine. The *degQ* gene is also a target of ComP-ComA regulation, but it is expressed at a very low level in the presence of glucose. This may reflect the fact that the regulatory cascade controlling late competence genes differs in some ways from that controlling *degQ* gene expression. Indeed, expression of late competence genes is strongly dependent on the products of *comL* and *comK* (44) while expression of *degQ* is not significantly affected by deletion of either of these genes (23a).

Regulation of *degQ'-'lacZ* expression by nutrient sources could be divided into three classes. (i) Regulation by phosphate depletion appears to be independent of regulation by ComP-ComA and DegS-DegU and involves sequences downstream from −78. A possible candidate involved in this regulation is the PhoR-PhoP two-component system (34, 35). (ii) Regulation by amino acid deprivation was found to be *comA* dependent and involves sequences downstream from −78. This suggests that amino acid deprivation is a signal triggering ComP-ComA-mediated control of *degQ* gene expression. Whether expression under these conditions is linked to the stringent response remains to be determined, for example, by examination of *degQ* gene expression in a *relA* strain (39). (iii) Catabolite repression and regulation by decoyinine involve sequences downstream from −40 and appear to be independent of both DegS-DegU and ComP-ComA. Expression of *degQ'-'lacZ* in the presence of decoyinine overrode catabolite repression. Previous work on the *citB* gene has suggested that treating cells with decoyinine inactivates or represses a carbon source-dependent negative regulator (9).

Finally, we examined whether the Spo0A effector, which belongs to the same family of regulatory proteins as DegU and ComA (40), had any effect on *degQ* gene expression. In contrast to the *degU* and *comA* mutations, a *spo0A12* mutation did not affect *degQ* synthesis (23a). This seems to indicate that the ComA and DegU effectors, although belonging to a complex network of two-component systems, are closely related, since they have several target genes in common, such as late competence genes and the *degQ* regulatory gene.

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REFERENCES

1. **Albano, M., R. Breitling, and D. A. Dubnau.** 1989. Nucleotide sequence and genetic organization of the *Bacillus subtilis comG* operon. J. Bacteriol. **171**:5386–5404.
2. **Albano, M., J. Hahn, and D. Dubnau.** 1987. Expression of competence genes in *Bacillus subtilis*. J. Bacteriol. **169**:3110–3117.
3. **Amory, A., F. Kunst, E. Aubert, A. Klier, and G. Rapoport.** 1987. Characterization of the *sacQ* genes from *Bacillus licheniformis* and *Bacillus subtilis*. J. Bacteriol. **169**:324–333.
4. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
5. **Aymerich, S., G. Gonzy-Tréboul, and M. Steinmetz.** 1986. 5′-Noncoding region *sacR* is the target of all identified regulation affecting the levansucrase gene in *Bacillus subtilis*. J. Bacteriol. **166**:993–998.
6. **Ayusawa, D., Y. Yoneda, K. Yamane, and B. Maruo.** 1975. Pleiotropic phenomena in autolytic enzyme(s) content, flagellation, and simultaneous hyperproduction of extracellular α-amylase and protease in a *Bacillus subtilis* mutant. J. Bacteriol. **124**:459–469.
7. **Chambert, R., and M.-F. Petit-Glatron.** 1984. Hyperproduction of exocellular levansucrase by *Bacillus subtilis*: examination of the phenotype of a *sacU*^{h} strain. J. Gen. Microbiol. **130**:3143–3152.
8. **Dubnau, D., and M. Roggiani.** 1990. Growth medium-independent genetic competence mutants of *Bacillus subtilis*. J. Bacteriol. **172**:4048–4055.
9. **Fouet, A., and A. L. Sonenshein.** 1990. A target for carbon source-dependent negative regulation of the *citB* promoter of *Bacillus subtilis*. J. Bacteriol. **172**:835–844.

9a. **Gibson, T. J.** 1984. Ph.D. thesis. University of Cambridge, Cambridge, England.

10. **Guillen, N., Y. Weinrauch, and D. A. Dubnau.** 1989. Cloning and characterization of the regulatory *Bacillus subtilis* competence genes *comA* and *comB*. J. Bacteriol. **171**:5354–5361.
11. **Henner, D. J., E. Ferrari, M. Perego, and J. A. Hoch.** 1988. Location of the targets of the *hpr-97*, *sacU32*(Hy), and *sacQ36*(Hy) mutations in upstream regions of the subtilisin promoter. J. Bacteriol. **170**:296–300.
12. **Henner, D. J., M. Yang, and E. Ferrari.** 1988. Localization of *Bacillus subtilis sacU*(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signaling systems. J. Bacteriol. **170**:5102–5109.
13. **Innis, M. A., K. B. Myambo, D. H. Gelfand, and M. A. D. Brow.** 1988. DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc. Natl. Acad. Sci. USA **85**:9436–9440.
14. **Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman.** 1989. Identification and characterization of genes controlled by the sporulation-regulatory gene *spo0H* in *Bacillus subtilis*. J. Bacteriol. **171**:4121–4129.
15. **Kraft, R., J. Tardiff, K. S. Krauter, and L. A. Leinwand.** 1988. Using mini-prep plasmid DNA for sequencing double stranded templates with Sequenase™. BioTechniques **6**:544–547.
16. **Kunst, F., M. Debarbouille, T. Msadek, M. Young, C. Mauel, D. Karamata, A. Klier, G. Rapoport, and R. Dedonder.** 1988. Deduced polypeptides encoded by the *Bacillus subtilis sacU* locus share homology with two-component sensor-regulator systems. J. Bacteriol. **170**:5093–5101.
17. **Kunst, F., M. Pascal, J. Lepesant-Kejzlarová, J.-A. Lepesant, A. Billault, and R. Dedonder.** 1974. Pleiotropic mutations affecting sporulation conditions and the synthesis of extracellular enzymes in *Bacillus subtilis* 168. Biochimie **56**:1481–1489.
18. **Lewandoski, M., and I. Smith.** 1988. Use of a versatile *lacZ* vector to analyze the upstream region of the *Bacillus subtilis spo0F* gene. Plasmid **20**:148–154.
19. **Mathiopoulos, C., and A. L. Sonenshein.** 1989. Identification of *Bacillus subtilis* genes expressed early during sporulation. Mol. Microbiol. **3**:1071–1081.
20. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Mitani, T., J. E. Heinze, and E. Freese.** 1977. Induction of sporulation in *Bacillus subtilis* by decoyinine or hadacidin. Biochem. Biophys. Res. Commun. **77**:1118–1125.
22. **Mohan, S., and D. Dubnau.** 1990. Transcriptional regulation of *comC*: evidence for a competence-specific transcription factor in *Bacillus subtilis*. J. Bacteriol. **172**:4064–4071.
23. **Msadek, T., F. Kunst, D. Henner, A. Klier, G. Rapoport, and R. Dedonder.** 1990. Signal transduction pathway controlling synthesis of a class of degradative enzymes in *Bacillus subtilis*: expression of the regulatory genes and analysis of mutations in *degS* and *degU*. J. Bacteriol. **172**:824–834.

23a. **Msadek, T., F. Kunst, A. Klier, and G. Rapoport.** Unpublished data.

24. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. **155**:335–350.
25. **Nagami, Y., and T. Tanaka.** 1986. Molecular cloning and nucleotide sequence of a DNA fragment from *Bacillus natto* that enhances production of extracellular proteases and levansucrase in *Bacillus subtilis*. J. Bacteriol. **166**:20–28.
26. **Nakano, M. M., M. A. Marahiel, and P. Zuber.** 1988. Identification of a genetic locus required for biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. J. Bacteriol. **170**:5662–5668.
27. **Nakano, M. M., and P. Zuber.** 1989. Cloning and characterization of *srfB*, a regulatory gene involved in surfactin production and competence in *Bacillus subtilis*. J. Bacteriol. **171**:5347–5353.
28. **Roggiani, M., J. Hahn, and D. Dubnau.** 1990. Suppression of early competence mutations in *Bacillus subtilis* by *mec* mutations. J. Bacteriol. **172**:4056–4063.
29. **Ruppen, M. E., G. L. Van Alstine, and L. Band.** 1988. Control of intracellular serine protease expression in *Bacillus subtilis*. J. Bacteriol. **170**:136–140.
30. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
33. **Schaeffer, P., J. Millet, and J.-P. Aubert.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54**:704–711.
34. **Seki, T., H. Yoshikawa, H. Takahashi, and H. Saito.** 1987. Cloning and nucleotide sequence of *phoP*, the regulatory gene for alkaline phosphatase and phosphodiesterase in *Bacillus subtilis*. J. Bacteriol. **169**:2913–2916.
35. **Seki, T., H. Yoshikawa, H. Takahashi, and H. Saito.** 1988. Nucleotide sequence of the *Bacillus subtilis phoR* gene. J. Bacteriol. **170**:5935–5938.
36. **Shimotsu, H., and D. J. Henner.** 1986. Construction of a single-copy integration vector and its use in analysis of regulation of the *trp* operon of *Bacillus subtilis*. Gene **43**:85–94.
37. **Shimotsu, H., and D. J. Henner.** 1986. Modulation of *Bacillus subtilis* levansucrase gene expression by sucrose and regulation of the steady-state mRNA level by *sacU* and *sacQ* genes. J. Bacteriol. **168**:380–388.
38. **Shyamala, V., and G. F.-L. Ames.** 1989. Amplification of bacterial genomic DNA by the polymerase chain reaction and direct sequencing after asymmetric amplification: application to the study of periplasmic permeases. J. Bacteriol. **171**:1602–1608.
39. **Smith, I., P. Paress, K. Cabane, and E. Dubnau.** 1980. Genetics and physiology of the *rel* system of *Bacillus subtilis*. Mol. Gen. Genet. **178**:271–279.
40. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
41. **Sullivan, M. A., R. E. Yasbin, and F. E. Young.** 1984. New shuttle vectors for *Bacillus subtilis* and *Escherichia coli* which

allow rapid detection of inserted fragments. Gene **29**:21–26.
42. **Tanaka, T., M. Kawata, Y. Nagami, and H. Uchiyama.** 1987. *prtR* enhances the mRNA level of the *Bacillus subtilis* extracellular proteases. J. Bacteriol. **169**:3044–3050.
43. **Trieu-Cuot, P., and P. Courvalin.** 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′5″-aminoglycoside phosphotransferase type III. Gene **23**:331–341.
44. **Van Sinderen, D., S. Withoff, H. Boels, and G. Venema.** 1990. Isolation and characterization of *comL*, a transcription unit involved in competence development of *Bacillus subtilis*. Mol. Gen. Genet. **224**:396–404.
45. **Wang, L.-F., and R. H. Doi.** 1990. Complex character of *senS*, a novel gene regulating expression of extracellular-protein genes of *Bacillus subtilis*. J. Bacteriol. **172**:1939–1947.
46. **Weinrauch, Y., N. Guillen, and D. A. Dubnau.** 1989. Sequence and transcription mapping of *Bacillus subtilis* competence genes *comB* and *comA*, one of which is related to a family of bacterial regulatory determinants. J. Bacteriol. **171**:5362–5375.
47. **Weinrauch, Y., R. Penchev, E. Dubnau, I. Smith, and D. Dubnau.** 1990. A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. Genes Dev. **4**:860–872.
47a. **Weinrauch, Y., and D. Dubnau.** Unpublished data.
48. **Yang, M., E. Ferrari, E. Chen, and D. J. Henner.** 1986. Identification of the pleiotropic *sacQ* gene of *Bacillus subtilis*. J. Bacteriol. **166**:113–119.
49. **Yang, M., H. Shimotsu, E. Ferrari, and D. J. Henner.** 1987. Characterization and mapping of the *Bacillus subtilis prtR* gene. J. Bacteriol. **169**:434–437.